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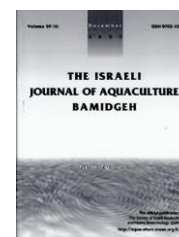


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## Effects of Dietary $\beta$ -glucan Supplementation on Growth, Innate Immune, and Capacity Against Pathogen *Streptococcus iniae* of Juvenile Pompano (*Trachinotus ovatus*)

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**Keywords:** Pompano; *Trachinotus ovatus*;  $\beta$ -glucan; immune; *Streptococcus iniae*

### Abstract

This study investigated the effect of dietary  $\beta$ -glucan on growth, immunity, and resistance against Streptococci in pompano *T. ovatus*. Pompano were fed a control diet, and diets which included 0.10% or 0.20% of  $\beta$ -glucan. After being fed the diets for 3-weeks, growth rate, survival, blood parameters, and immunity were examined, and the fish were challenged with *Streptococcus aniae* for 10 days. At the end of the feeding trial, RBC, WBC, WBC:RBC ratio, and lymphocytes significantly increased ( $P \leq 0.032$ ), while monocytes significantly decreased ( $P \leq 0.007$ ) in fish fed 0.10-0.20% of  $\beta$ -glucan. Moreover, lysozyme was significantly higher ( $P \leq 0.003$ ) in fish fed  $\beta$ -glucan, while the phagocytic ratio was only significantly higher ( $P = 0.037$ ) in fish fed 0.10%  $\beta$ -glucan. Furthermore, fish fed 0.10-0.20%  $\beta$ -glucan showed higher protection against *S. iniae* ( $P \leq 0.002$ ). Thus, a diet of  $\beta$ -glucan is recommended to improve non-specific immunity, growth in *T. ovatus*, and also to limit the adverse effects of *S. aniae* disease in this species.

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## Introduction

Pompano, *Trachinotus ovatus* (family Carangidae) is a highly valuable aquaculture species which is cultured in China, Singapore, Malaysia, and Vietnam (Chou and Lee, 1997; Do-Huu et al., 2016; Guo et al., 2018). This species has high economic value for the domestic and international market.

Pompano in aquaculture farms have occasionally endured outbreaks of diseases. At least, five pathogen species have been reported for *Trachinotus blochii*, including *Streptococcus* spp., *Escherichia coli*, *Salmonella* spp., *Pseudomonas* spp. and *Vibrio* spp. (Varol et al., 2014). *Streptococcus* are gram-positive bacteria, causing a systemic infection known as Streptococcosis (Eldar and Ghittino, 1999). Recently, *S. iniae* caused an outbreak in *T. ovatus* aquaculture in China (Guo et al., 2018). *Streptococci* including *Streptococcus agalactiae*, *S. iniae*, and *S. dysgalactiae* subsp. *dysgalactiae* were also isolated from pompano *T. ovatus* (Cai et al., 2016). Amongst the reported pathogens, *Streptococcus* species, *S. agalactiae*, and *S. iniae* have been mentioned more frequently than others (Cai et al., 2016). There is therefore an urgent need to find treatment for Streptococcosis in pompano.

In addition, Streptococcal disease is very difficult to control because at times it develops resistance to most chemicals and drugs (Klesius et al., 2008). Although a vaccine was successfully developed against streptococcal infection in fish, recent research reported that the *Streptococcus iniae* could re-appear even in vaccinated fish (Bachrach et al., 2001). In some cases, this pathogen was reported to be resistant to some antibiotics in pompano (Cai et al., 2016). The aim of this study was to try and find solutions that can enhance the sustainability of pompano aquaculture.

## Materials and Methods

This study complies with the ethical guidelines and regulations concerning animal use for experimentation and was carried out with the approval of the Institute of Oceanography, Vietnam Academy of Science and Technology (VAST), Vietnam for animal experimentation.

**Table 1.** Diet formulation for experimental and control diets

Ingredients (%)	D1	D2	D3
Fish meal (62% - Vietnam)	44.60	44.60	44.60
Gluten (wheat)	21.10	21.10	21.10
Soybean	10.40	10.40	10.40
Fish oil	3.60	3.60	3.60
Binder (inert)	1.10	1.10	1.10
Mineral premix	1.50	1.50	1.50
Vitamin premix	1.50	1.50	1.50
Corn starch	16.20	16.10	16.00
Macrogard® (β-glucan)	0	0.10	0.20
<b>TOTAL</b>	<b>100</b>	<b>100</b>	<b>100</b>

### Diet preparation and feeding

In this experiment, two doses of Macrogard® (1 and 2 g/kg) were incorporated in a basal diet formulated for pompano (crude protein: 48.6%; crude lipid: 6.7%). The formulation of a semi-purified diet for β-glucan and level of protein and lipid was referenced from other studies on pompano (Do-Huu et al., 2016). The supplemented levels of β-glucan were referenced from a previous study on pompano (Do-Huu et al., 2016; Do-Huu et al., 2018). All the ingredients were mixed, and water was slowly added to the ingredients to form a dough and passed through an extruder (2.5 mm die) to make pellets. The pellets were air dried and stored at -4°C until use. During the experimental period, fish were fed to apparent satiation twice daily (at around 8:30 and 17:30 h).

### Experimental fish

Healthy and disease-free pompano, *T. ovatus* of average body weight  $30.43 \pm 0.21$  g (average  $\pm$  SD) were obtained from a local hatchery and were acclimated for 2

weeks in a composite tank of 5 m<sup>3</sup> at the Marine Aquaculture Station at Institute of Oceanography, Nha Trang, Vietnam. During acclimation, fish were fed the basal diet at 4% of body weight and observed in satiation.

#### *Experimental tank and stocking*

After acclimation, twenty-four fish were stocked randomly into each 400 L-tank. A recirculation system with a flow rate of 400 L per minute was equipped in each tank. Fish were kept under natural photoperiod conditions and constant aeration throughout the trial. Water temperature was, 28-29.5°C; NH<sub>4</sub><sup>+</sup>/NH<sub>3</sub>, < 0.04 mg/L; NO<sub>2</sub><sup>-</sup>: 0.01 mg/L and pH, 8.0-8.3.

#### *Experimental design*

The experiment was designed to evaluate non-specific immune, growth and mortality of fish fed different  $\beta$ -glucan levels and tolerance against *Streptococcus iniae* infection. There were six replicates for each diet treatments: a control basal diet, 0.10% and 0.20% of  $\beta$ -glucan supplementation diets. Fish were fed  $\beta$ -glucan supplemented diets or control diet for 3 weeks (stage 1) and then challenged against *Streptococcus iniae* and mortality was recorded for 10 days (stage 2). The fish were fed the same corresponding diet during the disease infection test.

#### *Sampling and data collection*

After stage one, (Day 21, end of feeding trial), all fish were weighed, and three fish per tank were collected for hematological determination and immune analysis. Prior to weighing and sampling, feed was not served for 24 hours. Fish were randomly collected and euthanized with 100 mg/L tricaine methanesulfonate (MS-222, Sigma, USA) and blood samples withdrawn with 1 mL syringes. A subdivision of the collected blood was placed into tubes with heparin as an anticoagulant to analyze the total erythrocyte and leucocyte count. The rest of the blood was placed in Eppendorf tubes and kept in ice, allowing the blood to clot for 2-4 h, followed by centrifuging for 10 min. at 4000 g to collect serum. The serum was then stored at -80°C for later lysozyme analysis. After withdrawing blood, the head kidney was taken for analysis of phagocytic activity.

#### *Hematological assays*

Determination of leukocytes (WBC) and Erythrocytes (RBC) was conducted using a hemocytometer (Blaxhall and Daisley, 1973). Differential leukocyte counts including monocytes, lymphocytes, neutrophils, and eosinophils, were obtained by blood smears, air-dried, methanol fixed, and followed by staining in Diff-Quick®. Leucocytes were classified relying on the morphology and counted under a microscope at a magnification of 1000  $\times$ .

#### *Isolation of leucocyte from head kidney*

Macrophages from head kidney were isolated following the method described by Secombes (1990). Briefly, the dissected head kidney was cut in small pieces and placed in RPMI-1640 medium supplemented with 100 IU/mL streptomycin, 100 IU/mL penicillin, 10 IU/mL heparin and 2% fetal calf serum (FCS), followed by grinding through a 100  $\mu$ m mesh. Then, 34%/51% Percoll was added to the leucocyte cell suspension and centrifuged for 35 min at 1000 g (4°C). Subsequently, leucocyte cell interphase was collected and washed twice by using PBS, followed by centrifuging at 200 g (10 min). Viable cell levels were determined using Trypan blue (0.1%) and the cell density was checked by a hemocytometer. Cells were then suspended in L-15 medium with 0.1% FCS added to adjust the density to 1  $\times$  10<sup>7</sup> cells/mL for analysis.

#### *Phagocytic activity*

The phagocytosis assay of pompano leukocytes was performed according to the method of Siwicki et al. (1994) with a slight modification. The 100  $\mu$ L cells of head kidney leucocytes (1  $\times$  10<sup>7</sup> cells/mL) were placed in the sterile slide (3 replicates slide for each fish) for ~30 min (at 25°C). Then, 100  $\mu$ L zymosan suspension (Sigma) 1  $\times$  10<sup>8</sup> cells/mL were added, mixed then incubated for 1 h at 25°C. The slide was washed by PBS to remove unattached cells. After drying at room temperature, the slide was fixed with methanol and stained with 1 mL Congo Red for 5 min. A total of about five hundred phagocytes per slide were observed under a microscope under oil immersion at 1000  $\times$ .

magnification. The phagocytic activity was estimated as the phagocytic ratio (PR, %) = nr. of phagocytic cells with engulfed zymosan/nr. of phagocytic cells (Sahoo and Mukherjee, 2001).

#### *Serum lysozyme activity*

To determine lysozyme, 25  $\mu$ L of collected serum was microplated with 3 well replicates for each, and PBS was used as blank. Then, hen egg lysozyme dilutions of (0 - 20  $\mu$ g/mL; Sigma-Aldrich) were used to create a standard curve. Then, 175  $\mu$ L of the *Micrococcus lysodeikticus* (0.75 mg/mL). Next, the microplate was mixed at 25°C. The difference in turbidity was recorded by a microwell plate reader 440 nm at 15 min and 30 min (Ellis, 1990).

#### *Streptococcus challenge trial*

The *Streptococcus iniae* bacteria were provided by Nha Trang University. Three weeks post diet feeding, eighteen fish from each diet treatment were randomly collected and injected with *Streptococcus iniae* (stage 2). Each fish was injected intraperitoneally with 100  $\mu$ L suspension of *Streptococcus* ( $1.5 \times 10^4$  cfu/fish) in PBS using a 0.3 mL insulin syringe. One treatment was held in three 400 L-tanks as replicates. The injection dose of bacteria is a lethal dose 50% (LD<sub>50</sub>) as described in below. For negative control, ten fish from the group of fish fed the control diet were collected and injected with 100  $\mu$ L saline water, and these fish were kept in separately to avoid cross infection. The fish in each diet treatment were given the same assigned diets during the challenge test with adjustment for satiation by observation. Fish mortality was recorded twice a day for 10 days. It was assumed that the longer a fish survived, the greater was its resistance to *Streptococcus iniae* infection.

#### *Determination of lethal dose 50% (LD<sub>50</sub>)*

The lethal dose, LD<sub>50</sub> at ten days was determined by intraperitoneal injecting 100  $\mu$ L inoculum per fish with graded doses of *Streptococcus iniae* ( $10^2$ ,  $10^3$ ,  $10^4$ ,  $10^5$ ,  $10^6$  and  $10^7$  cfu/fish) at 10 fish for each dose. The dose of  $1.5 \times 10^4$  cfu/fish was selected for the challenge test for ten days in this experiment.

#### *Data calculation and statistical analysis*

Specific Growth rate (SGR %/d), survival rate, and relative percent survival (RPS) was calculated as follows:

$SGR = 100 \times [\ln(W_t) - \ln(W_o)]/\text{days}$ ;  $S = 100 \times (n_t/n_o)$ ;  $RPS = 100 \times (1 - M_d / M_o)$  (Amend, 1981),

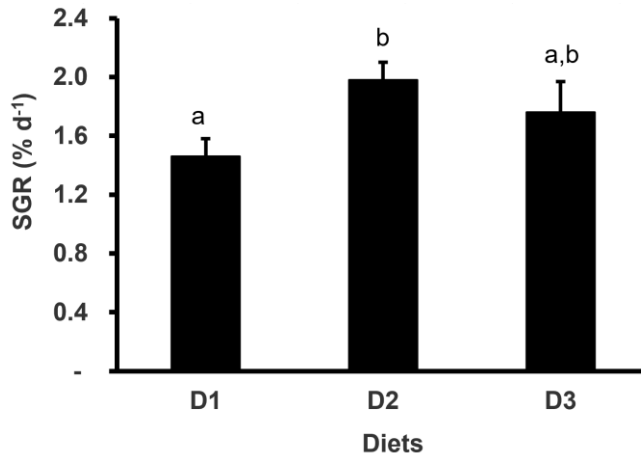
where  $W_t$  and  $W_o$  were the weight of the fish at time  $t$ ; and at the commencement;  $S$ : Survival rate (%),  $n_t$  and  $n_o$  were the number of the fish at time  $t$  and at the commencement,  $M_d$  and  $M_o$  were the percentage mortalities of fish on  $\beta$ -glucan diets and control diet treatments, respectively.

ANOVA was applied to compare average growth and survival value among diet treatments. Data were tested before analysis. The post hoc test (LSD) was used where significance was detected. Cumulative survival curves of *Streptococcus* infected fish fed dietary  $\beta$ -glucan levels compared to fish fed control diet were determined by Kaplan-Meier followed by log-rank test. Relative risk, hazard ratio between diets were also analyzed.  $P < 0.05$  was considered significant in each case. All statistics were performed using SPSS 18.

## **Results**

### *Growth performance of pompano fed dietary $\beta$ -glucan*

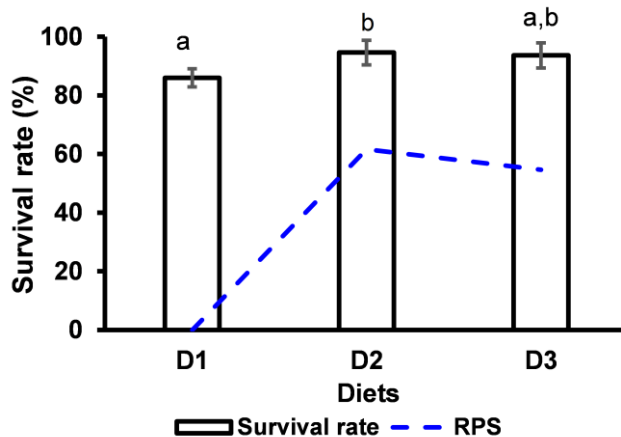
Initial mean weight of pompano was uniform among the treatments, with no significant difference (ANOVA,  $P = 0.842$ ). Fish weight reached over 35 g at the end of the feeding trial (Day 21). After 21-days of diet feeding, the specific growth rate (SGR) of pompano fed 0.10% (D2) was higher than SGR of fish fed control diet ( $P = 0.010$ ). However, there was no significant difference ( $P \geq 0.221$ ) either between SGR of fish fed D1 and fish fed D3 or between fish fed diet D2 and D3. (Fig. 1)



**Fig. 1.** Growth performance of pompano fed dietary  $\beta$ -glucan supplementation. Data are presented as the means  $\pm$  SD. Different letters indicate significant difference between treatments.

#### *Survival rate and relative survival rate (RSR) of pompano*

At the end of the feeding trial (Day 21), survival rates of fish ranged from 86.01% (in the fish fed control diet) to 94.63% (in the fish fed diet with 0.10%  $\beta$ -glucan, D2) (Fig. 2). Survival rate was highest in the pompano group fed the diet supplemented with 0.10%  $\beta$ -glucan and was significantly higher than the survival of the control group of pompano ( $P = 0.001$ ). However, the survival of fish fed the diet supplemented with 0.20%  $\beta$ -glucan (D3) did not significantly differ from the survival rates of the control group of fish or fish fed diet with 0.10%  $\beta$ -glucan (D2) ( $P \geq 0.120$ ). Relative survival percentages were 60.6% and 54.6% for groups of fish fed diets supplemented with 0.10% (D2) and 0.20%  $\beta$ -glucan (D3), respectively (Fig. 2).



**Fig. 2.** Survival rate and relative percentage survival (RPS) of pompano fed different levels of dietary  $\beta$ -glucan. Data are presented as the means  $\pm$  SD. Different letters indicate significant difference between treatments.

#### *Hematological parameters of pompano fed dietary β-glucan supplementation*

There was a significant difference in the hematological index between the dietary treatments. White blood (WBC) and red blood cells (RBC) of pompano fed both levels of Macrogard<sup>®</sup> were higher than the fish fed control diet ( $P \leq 0.004$ ), but there were no differences ( $P \geq 0.249$ ) between them (Table 2). Ratios of WBC:RBC were also higher in fish fed diet supplemented with 0.10% (D2) and 0.20%  $\beta$ -glucan (D3) compared to fish fed control diet ( $P \leq 0.012$ ), but no significance ( $P \geq 0.564$ ) in the WBC:RBC ratio among the fish fed two levels of  $\beta$ -glucan (Table 2). The leukocytes, lymphocytes, and monocytes altered significantly in fish fed dietary  $\beta$ -glucan supplementations compared to fish fed control. Pompano fed the diet with 0.10% (D2) and 0.20%  $\beta$ -glucan (D3) inclusion showed significantly higher proportions of lymphocytes, in comparison to fish fed control ( $P \leq 0.032$ ). In contrast, monocytes were significantly reduced in fish fed  $\beta$ -glucan in the diet ( $P \leq 0.007$ ). However, lymphocytes and monocytes were not different between fish fed diets supplemented with 0.10% (D2) and 0.20%  $\beta$ -glucan (D3) ( $P \geq 0.363$ ). In addition, dietary  $\beta$ -glucan showed no significant influence on neutrophil and

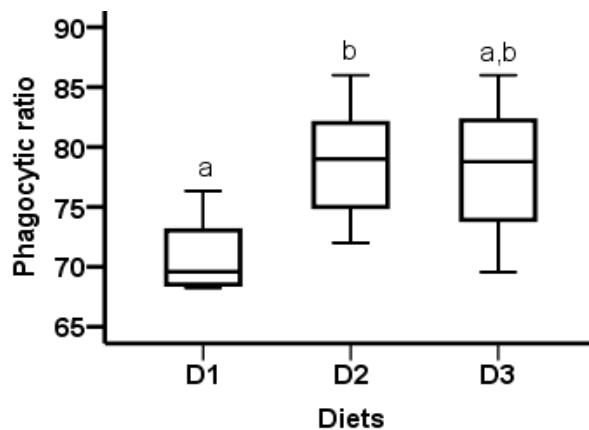
basophil cell counts ( $P \geq 0.104$ ). However, eosinophil was significantly lower ( $P = 0.022$ ) in fish fed 0.10% (D2) compared to fish fed control (Table 2).

**Table 2.** Hematological parameters of pompano after 3-weeks of diet feeding. Different letters indicate significant difference between treatments at each time point.

	Diets		
	D1	D2	D3
RBC ( $\times 10^6 \text{ mm}^{-3}$ )	$4.41 \pm 0.11^a$	$5.22 \pm 0.08^b$	$5.16 \pm 0.17^b$
WBC ( $\times 10^5 \text{ mm}^{-3}$ )	$0.88 \pm 0.05^a$	$1.23 \pm 0.07^b$	$1.23 \pm 0.09^b$
Monocyte (%)	$19.90 \pm 1.68^a$	$14.05 \pm 1.76^b$	$12.51 \pm 0.49^b$
Lymphocyte (%)	$66.60 \pm 1.96^a$	$73.49 \pm 1.12^b$	$73.77 \pm 1.45^b$
Neutrophil (%)	$9.81 \pm 0.62$	$10.10 \pm 1.01$	$10.82 \pm 1.13$
Eosinophil (%)	$2.76 \pm 0.69^a$	$0.91 \pm 0.32^b$	$1.75 \pm 0.54^{ab}$
Basophil (%)	$0.95 \pm 0.66$	$1.45 \pm 0.40$	$1.15 \pm 0.56$
WBC : RBC (%)	$0.20 \pm 0.02^a$	$0.28 \pm 0.01^b$	$0.26 \pm 0.02^b$

#### *Phagocytic ratio of pompano*

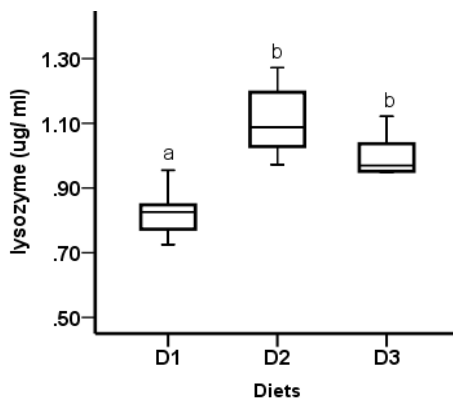
There were significant increases in the phagocytic ratios (PR) of pompano fed the diet D2 (0.10%  $\beta$ -glucan) compared to PR value in fish fed control diet (D1) ( $P = 0.037$ ), while no statistical significance was observed in PR of fish fed diet D3 (0.20%  $\beta$ -glucan) compared to control ( $P = 0.059$ ). Also, the PR values were not significantly different ( $P = 0.531$ ) between fish fed diets D2 and D3. (Fig. 3).



**Fig. 3.** The phagocytic ratio (PR) in pompano fed different levels of  $\beta$ -glucan. Data are presented as the means  $\pm$  SD. Different letters indicate significant difference between treatments.

#### *Lysozyme activity of pompano fed dietary $\beta$ -glucan*

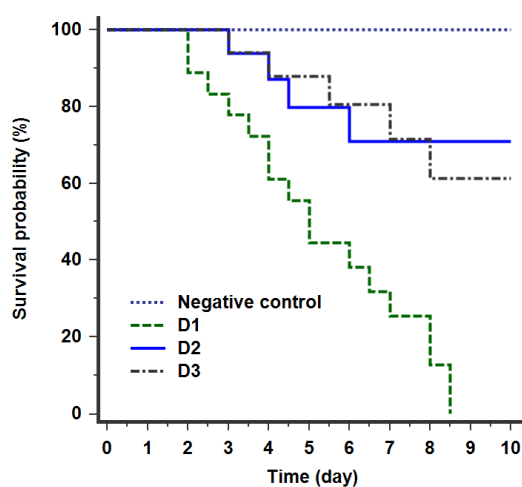
Post-3-weeks of diet feeding, dietary  $\beta$ -glucan supplementation significantly enhanced serum lysozyme activity in pompano. The highest level of lysozyme was in fish fed diet D2, followed by the fish fed diet D3, which were both significantly higher than lysozyme activity in fish fed the control ( $P \leq 0.003$ ). However, no significant difference ( $P = 0.053$ ) was found in lysozyme activities of fish fed diets D2 (0.10%  $\beta$ -glucan) and D3 (0.20%  $\beta$ -glucan). (Fig. 4)



**Fig. 4.** Serum lysozyme activity of pompano fed different levels of dietary  $\beta$ -glucan supplementation. Data are presented as the means  $\pm$  SD. Different letters indicate significant difference between treatments.

*Cumulative mortalities, relative risk, and hazard ratio of pompano challenged with *Streptococcus iniae**

After 21 days of feeding with  $\beta$ -glucan supplemented diets, the pathogen challenge test was performed for 10 days. The accumulative survival rate was determined on the 10<sup>th</sup> day and is presented in Fig. 5. During the infection period, there were no mortalities in the negative control group. After challenging with *Streptococcus*, the first mortality was recorded after 2 days in the control group, while in fish fed diet 0.10% and 0.20%  $\beta$ -glucan the first mortality occurred on day 3, respectively. After 10 days, the highest survival rate was observed in fish fed 0.10%, followed by fish fed 0.20%  $\beta$ -glucan inclusion in the diet, which were both significantly higher ( $P \leq 0.002$ ) than fish fed control diet (D0.00). The survival rates of fish fed diets supplemented with 0.10% and 0.20%  $\beta$ -glucan did not significantly differ ( $P = 0.850$ ) during the challenge period (Fig. 5). When exposed to pathogen, the hazard ratio of fish fed diets supplementation of 0.1% and 0.2% were 0.216 (95% CI: 0.0873 to 0.5327) and 0.223 (95% CI: 0.0912 to 0.5453), respectively.



**Fig. 5.** Survival curves of juvenile pompano fed  $\beta$ -glucan diets and challenged with *Streptococcus*

### Discussion

To our knowledge, this is the first finding showing the efficiency of dietary  $\beta$ -glucan supplementation on boosting the resistance of pompano against *Streptococcus iniae* pathogen infection. Also, the growth and hematological index and immune response were higher in fish fed  $\beta$ -glucan diet.

In line with the previous study (Do-Huu et al., 2016), the present study confirmed that the growth and survival of pompano were significantly improved when their diet was supplemented with 0.10%  $\beta$ -glucan. This is also in agreement with another study which revealed that dietary  $\beta$ -glucan could improve growth in aquaculture species including red sea bream *Pagrus major* (Dawood et al., 2017). In contrast,  $\beta$ -glucan has shown no effects on the growth of European sea bass *Dicentrarchus labrax* (Bagni et al., 2005). Possible factors are the concentration of  $\beta$ -glucan administration in the diet, species-specific response, and experimental conditions as well as the administration time (Do-Huu et al., 2016). In the present study, juvenile pompano grew rapidly and the significant differences were detected between diet treatments after 21-days of feeding. Research reveals that fast growth rate may result from the energetic benefits obtained through  $\beta$ -glucan administration (Ai et al., 2007), manipulation of intestinal microbiota (Do-Huu et al., 2016), or digestive enzymes produced by beneficial bacteria (Wu et al., 2014).

In this study, total leucocyte count increased in the fish fed  $\beta$ -glucan supplemented diets. Similarly to our previous study (Do-Huu et al., 2016), the present study showed that lymphocyte and WBC increased significantly in pompano, *T. ovatus*, supplied 0.10%  $\beta$ -glucan in the diet. In agreement with our results, lymphocyte and WBC



increased in Persian sturgeon fish *Acipenser persicu* fed dietary  $\beta$ -glucan (Aramli et al., 2015).

In the innate immune system, fish blood lysozyme was also proposed as a valuable indicator to reflect the bio-defense level and in evaluating the influences of immunostimulants (Siwicki and Anderson, 1993). Also, lysozyme is a significant defense enzyme, which exists in the blood and lymphoid tissues of fish. The enzyme plays a vital role in fish health and is one of the most important natural resistances in fish protection against microbial invasion (Saurabh and Sahoo, 2008). It was also reported that  $\beta$ -glucan administration resulted in increase in serum lysozyme, phagocytic activity, and induced macrophages hyperplasia, enhanced state of host defense against disease (Kokoshis et al., 1978). Both lysozyme and IgM activities can reflect the immune function of fish in alternative environments or nutritional conditions (Lin and Shiau, 2003). Additionally,  $\beta$ -glucan administration was reported to stimulate lysozyme, phagocytic activity, antibody production and respiratory bursts of many species such as sea bass *Dicentrarchus labrax* (Bagni et al., 2005). Furthermore, dietary  $\beta$ -glucan supplementation significantly enhanced lysozyme activity of large yellow croaker *Pseudosciaena crocea* (Ai et al., 2007). The result of this study is in line with other findings that increasing lysozyme by dietary  $\beta$ -glucan suggesting that the  $\beta$ -glucan might contain some bioactive polysaccharide involving in regulating the host immune response (Kokoshis et al., 1978).

Studies have indicated that streptococcal diseases are very difficult to control because they are resistant or have become resistant to most chemicals and drugs (Cai et al., 2016). In addition, although vaccine was applied to treat streptococcal infection in fish, the study has shown that the *Streptococcus* pathogen could recover again even in vaccinated fish (Bachrach et al., 2001). The treatment for streptococcal disease in pompano is still not fully determined (Cai et al., 2016). Results in this study suggest that  $\beta$ -glucan diet could stimulate some degree of immunity in pompano. Dietary  $\beta$ -glucan supplementation at 0.10-0.20% in the diet of pompano was significantly associated infection of *S. aniae* risk, presenting a significantly lower risk when challenged against *Streptococcus* in comparison to fish fed basal diet. Therefore, the present findings can be beneficial for farmers by reducing the use of antibiotics in aquaculture and improving sustainability. However, further research should be conducted on different sized fish, using different doses, and adjusting administration periods.

Research has also revealed that dietary  $\beta$ -glucan administration increases resistance to other disease infection in aquaculture fish. In fact, dietary  $\beta$ -glucan has shown to significantly increase survival of *Pseudosciaena crocea* challenged with *Vibrio harveyi* (Ai et al., 2007) and in *Labeo rohita* fingerlings against *Edwardsiella tarda* and *Aeromonas hydrophila* infection (Misra et al., 2006). In line with these findings, our results show that dietary  $\beta$ -glucan promoted higher resistance of pompano to *S. iniae* pathogen infection together with elevated immune response.

In conclusion, the present findings confirm the supplementation of  $\beta$ -glucan in the diet is beneficial in boosting non-specific immunity, growth performance, survival rate, and tolerance to *Streptococcus iniae* infection of pompano *T. ovatus*. The addition of 0.10% of  $\beta$ -glucan to the pompano diet is recommended to boost disease resistance, immunity, and growth performance. However, further potential benefits of  $\beta$ -glucan application such as boosted resilience other infectious pathogens should be explored. In addition, the influence of dietary  $\beta$ -glucan on different life stages such as larval and broodstock may be tested experimentally.

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